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BRIEFER ARTICLES

THE USE OF CELLOIDIN MEMBRANES FOR THE DEMONSTRATION OF OSMOSIS

(WITH THREE FIGURES)

Although the value of a celloidin film as an osmotic membrane is well known to botanists, little use of it has been made on account of the difficulties experienced in manipulation. A description of the method heretofore employed can be found in most textbooks on bacteriology. In this method, a celloidin solution is poured into a test tube or an Erlenmeyer flask and carefully rotated until a film which can be lifted out is formed on the inside of the tube, the sac thus formed being used as the membrane. Another method of preparing celloidin membranes, first described by BIGELOW and GEMBERLING,[1] is generally unknown to botanists, although considerable use has been made of it by chemists. This method consists in pouring a celloidin solution on a clean mercury surface from which the membrane thus formed is removed after the celloidin has sufficiently hardened. I have found that a three-inch Petri dish forms a very convenient container for the mercury. After carefully cleaning the surface of the mercury, enough 10 per cent celloidin solution is poured on it to cover an area 3–4 cm. in diameter. In 2–5 min. the ether and alcohol have evaporated sufficiently to allow the membrane to be lifted. It is important that the celloidin film be "ripe" before attempting to lift it; otherwise a stringy mass will be formed instead of a sheet. As soon as its edge appears scalloped and the surface shows small polygonal patches, the film can be raised. Its general appearance at the time it is "ripe" enough to be taken off is shown in fig. 1.

The best support for the osmotic membrane prepared as described above is the bulb of a thistle tube having a stem a half-inch in length, a thistle tube with a shortened stem being much more easily filled. It can be held in a test tube rack or in a small-necked bottle with the opening of the bulb uppermost and the celloidin membrane placed over its open end (fig. 2, *A*). The overlapping portion of the membrane should then be pressed to the sides of the flange of the bulb and tied securely with stout Manila twine. As the membrane dries it adheres tightly to the flange, and since it contracts somewhat on drying, it becomes stretched

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[1] BIGELOW, S. L., and GEMBERLING, A., Collodion membranes. Jour. Am. Chem. Soc. **29**:1576–1599. 1907.

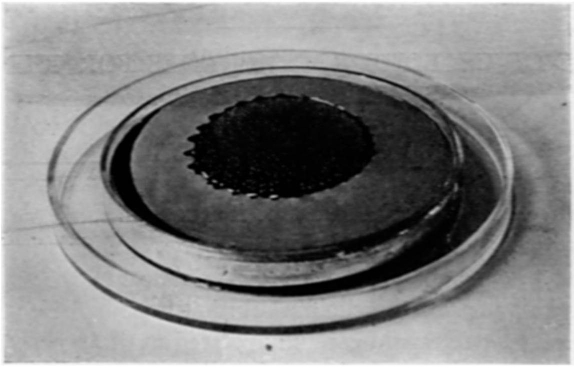

FIG. 1.—Celloidin film on a dish of mercury; the edges show the characteristic appearance at time when it is "ripe."

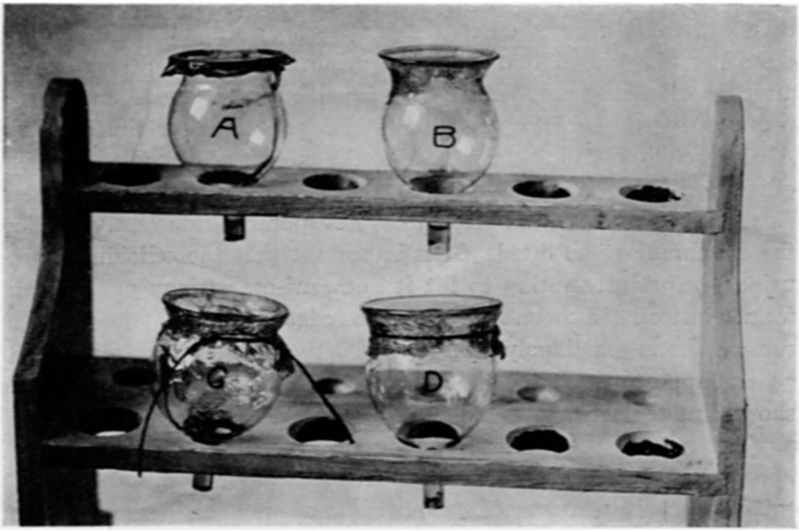

FIG. 2.—Different stages in the formation of celloidin membranes: *A*, membrane after being placed over the bulb of a thistle tube; *B*, showing how the film adheres to the flange; *C*, after tying the membrane on the thistle tube; *D*, a double membrane.

as taut as the head of a drum (fig. 2, *B*). The ends of the twine are then cut off and the part of the film which extends more than a quarter of an inch beyond the twine should be trimmed off with a sharp knife (fig. 2, *C*). This trimmed membrane after further drying for an hour should then be kept in a dish of water until needed for use. MATHEWS[2] dried his membranes over a steam coil for several days, but found that perfectly dry membranes did not allow the solute to pass through very rapidly.

As considerable variation was found in membranes made in the same manner and at the same time, various attempts were made to reinforce them and make their tensile strength more uniform. The method used for testing a membrane consisted in suspending the osmometer and then pouring mercury into the tube until the membrane broke, noting the height of the column of mercury in the tube at that time. Usually there was not a rupture of the entire membrane, but a small leak in one place, and that generally near the flange of the bulb. The different methods used in making the membranes, and their tensile strength, as indicated by the height of the mercury column, are shown in the table given below.

TABLE SHOWING THE RELATIVE STRENGTH OF VARIOUS MEMBRANES

The figures refer to the height (in cm.) of the column of mercury when the membrane broke.

Method of formation	1st	2d	3d	4th	5th	Average
Not tied; put in water 5 min. after being made	13.5	8.0	14.0	......	......	11.83
Not tied; air dried one hour, then put in water	10.5	4.5	11.5	16.0	......	10.39
Membrane reinforced with bar netting; not tied	17.0	12.5	19.0	......	......	16.17
Not tied; hardened in chloroform 48 hours	15.0	12.5	15.0	......	......	14.17
Double membrane; not tied	73.0	29.0	73.5	54.0	64.0	58.70
Single membrane, tied; air dried 24 hours	9.0	59.0	11.0	8.5	41.0	25.70
Same, then soaked 24 hours in water	27.0	8.0	62.0	52.0	22.0	34.20
Double membrane, tied and dried in air an hour	94.0	36.0	238.0	230.0*	215.0*	163.40

* No break in the membrane occurred when the height of the mercury column had reached this point.

As the above tests show, the double membranes are by far the strongest. These are produced by making a second single membrane and superimposing it over one made in the manner described above (fig. 2, *D*). There may be one or two bubbles of air between the two membranes, but these do not seem to interfere with the work or the strength of the membrane.

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[2] MATHEWS, J. H., Osmotic experiments with collodion membranes. Jour. Phys. Chem. **29**:281–291. 1910.

In three days an osmometer made in this manner and filled with a 25 per cent cane sugar solution, under ordinary laboratory conditions, will send up a column of liquid 130–197 cm. high. When similar osmometers are filled with a 10 per cent sodium chloride solution, the liquid will rise 138–172 cm. in the same length of time and under the same conditions. At the end of this time the water column begins to sink slowly.

Attempts were made to render membranes semipermeable by using tannic acid and copper ferrocyanide. Osmometers having membranes prepared as described above were filled with a 5 per cent tannic acid solution and then set in dishes containing the same solution. Enough melted 10 per cent gelatin solution to form a film was poured in the bottom of other osmometers, allowed to harden, and then treated with the 5 per cent tannic acid as before. After standing in the tannic acid solution for 48 hours, the osmometers were placed in water to soak out the excess of tannic acid. The acid leeched out for a long time afterward. In some cases the leeching continued for as long as three weeks, even after several changes of water. On account of the difficulty of freeing the membranes of the excess of tannic acid, no further experiments were made with this substance.

For the formation of the copper ferrocyanide membranes, M/20 solutions of copper sulphate and potassium nitrate were used. Osmometers were filled with the copper sulphate solution and immersed in the potassium ferrocyanide solution, while others containing potassium ferrocyanide were placed in the copper sulphate solution (fig. 3). The latter method proved the better, as PFEFFER[3] found in his experiments with porous clay cups. By the end of the third day the celloidin film becomes impregnated with the copper ferrocyanide. There may be some exosmosis of the potassium ferrocyanide and a consequent formation of copper ferrocyanide on the outside of the membrane, but this is easily washed off with water. The precipitation membrane is not formed uniformly throughout the celloidin, but has a mottled appearance at first. Later the membrane becomes uniform in color and texture.

These membranes need not be used at once, but should be used within a month. It was found by BIGELOW and GEMBERLING that for celloidin membranes standing in water for three months the permeability is considerably decreased. The rapidity of the passage of the liquid through the membranes and its consequent rise in the osmometer varies considerably, the osmometers with the smaller, dryer membranes showing a much slower rise. Membranes with a large osmotic surface, for example 5 cm. in diameter, will allow the liquid to rise quite rapidly.

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[3] PFEFFER, W., Osmotische Untersuchungen. Leipzig. 1877.

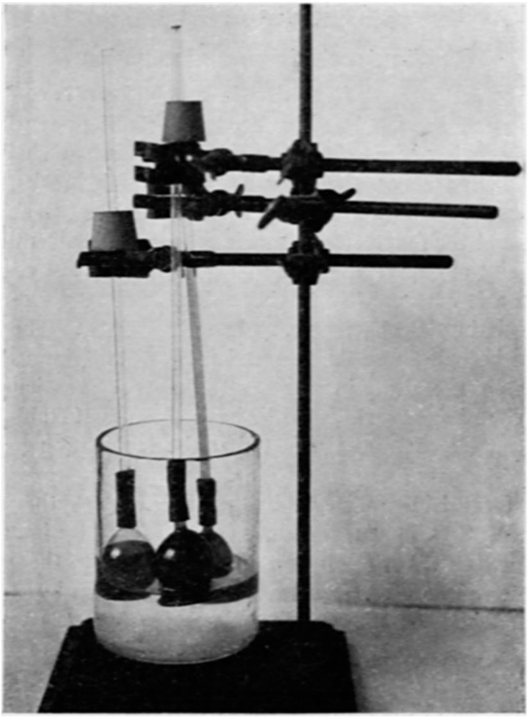

FIG. 3.—Osmometers filled with potassium ferrocyanide solution and immersed in a copper sulphate solution; the precipitation membrane is being formed inside of the celloidin film.

When an osmometer prepared in this manner is set up, water will rise to a much greater height than in those having untreated celloidin membranes. In one experiment with this type of membrane the water rose 8 meters.

I am indebted to Professor J. B. OVERTON for advice and criticism during the progress of this work, for which I take this opportunity of expressing my appreciation.—GILBERT MORGAN SMITH, *University of Wisconsin, Madison, Wisconsin.*